POPULATION STRUCTURE IN AN INDIAN COOPERATIVE SPIDER, *STEGODYPHUS SARASINORUM* KARSCH (ERESIDAE)

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ABSTRACT.   Twenty-nine colonies of a population of the cooperative spider *Stegodyphus sarasinorum* Karsch (Eresidae), from two sites in Bangalore, Karnataka State, India, were examined using protein allozyme electrophoresis. Thirty-five enzyme systems were examined. Twenty-two enzymes (the products of 25 putative loci) gave scorable results. Lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were polymorphic with two alleles each; hexokinase exhibited uninterpretable variation. The rest were monomorphic. One LDH allele was found at only one of two collection sites, and one G6PDH allele was found only at the other collection site. The pattern of variation in *S. sarasinorum* is similar to that found in three other studies of cooperative spiders: extreme population subdivision, with most colonies consisting of identical homozygotes.

Stegodyphus sarasinorum Karsch (1891) is one of three permanently cooperative species in the genus *Stegodyphus* (Kraus & Kraus 1988). *S. sarasinorum* has been found in India, Sri Lanka, Nepal and Afghanistan (Kraus & Kraus 1988). The natural history of *S. sarasinorum* has been investigated by Jambunathan (1905), Bradoo (1972, 1975, 1980), Jacson & Joseph (1973), and Kraus & Kraus (1988). Individuals live in large cooperatively built colonies with a nest or retreat constructed of silk woven together with leaves, twigs, and remains of food, and a sheet web for prey capture (Jacson & Joseph 1973). Prey capture is also a cooperative effort and when the prey has been subdued many spiders may join in the feeding, even those who did not participate in the actual prey capture activities (Bradoo 1980). The females care for young during the first few instars by feeding the spiderlings (Bradoo 1972). When the young become old enough they begin capturing their own prey and sometimes feed upon older females that die within the nest (Bradoo 1972). Like other cooperative spiders (Pain 1964; Darchen 1967; Jackson & Smith 1978; Fowler & Levi 1979; Avilés 1986; Rowell & Main 1992), *S. sarasinorum* colonies exhibit strongly female-biased sex ratios with 0.15 to 0.28 males for every female (Jacson & Joseph 1973). These spiders are tolerant of individuals from other nests (Kullman 1968), and migration among colonies in close proximity has been observed (Bradoo 1972).

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Little is known about dispersal, population structure or mating in *S. sarasinorum*. Another cooperative species of *Stegodyphus*, *S. mimosarum*, is known to balloon (Wickler & Seibt 1986). Jambunathan (1905) and Jacson & Joseph (1973) both record ballooning by immatures of *S. sarasinorum*, but this has not been examined to determine ballooning's importance in the dispersal or colony foundation of *S. sarasinorum*. Bradoo (1972) reports that new colonies were established by spiderlings that left the main colony to form daughter colonies connected to the mother nest by common web sheets. Bradoo (1980) reports that new colonies were also formed by gravid females who had left the original nest and formed new nests in which to keep their cocoons.

In this study we used protein allozyme electrophoresis to examine genetic variation within and among colonies of *S. sarasinorum* collected from two sites in south central India.

METHODS

Collection.—Colonies of *S. sarasinorum* were collected by DRS in August, 1990 from two localities in Bangalore, Karnataka State, India. Colonies A1, A2, B1, B2 and E were collected from the campus of G. K. V. K. Agricultural University. Spiders from colonies A1 and A2 were accidentally allowed to travel from one colony to the other after collection, so individuals from A1 and A2 were scored as members of one colony. Colonies in the series labeled C and D were collected from the grounds of the Indian Institute of Science (I. I. S.). Colonies D4a through

D4d shared capture webs, forming a colony cluster. Spiders were removed from the colony webbing and frozen in liquid nitrogen. Spiders were stored at −80 °C until they were used in allozyme studies.

Electrophoresis.—Individual spiders were assayed for allozyme variation by electrophoresis on cellulose acetate plates (Helena Laboratories, Beaumont, Texas). Specimens were crushed in 100 μl of extraction buffer (0.1 M tris, pH 7.0, with 40 mg EDTA, 20 mg NAD, 10 mg NADP, and 250 μl of β-mercaptoethanol per 100 ml: Hagen et al. 1988). Specimens were kept chilled during the crushing to minimize enzyme degradation. Using an applicator device (Super-Z Bar, Helena Laboratories) one μl aliquots from each specimen were applied to cellulose acetate plates which had been hydrated by soaking in running buffer for at least ten min. The plate was then placed in a gel rig (Zip Zone Electrophoresis Chamber, Helena Laboratories) and a constant 160 volts was applied at room temperature for 35 min. Five running buffers (Richardson et al. 1986) were tried to obtain the best resolution of the protein bands. These were: 10 mM Na_2HPO_4, 2.5 mM citric acid, pH 6.4 ("A"); 11.6 mM Na_2HPO_4, 8.4 mM NaH_2PO_4, pH 7.0 ("B"); 50 mM tris, 20 mM maleic acid, pH 7.8 ("C"); 15 mM tris, 5 mM EDTA, 10 mM $MgCl_2$, 5.5 mM boric acid, pH 7.8 ("D"); and 25 mM tris, 192 mM glycine, pH 8.5 ("I"). Following the run, enzyme specific stains (Richardson et al. 1986) were mixed with 1.5% agar and applied to the plates. After stains had fully developed, the agar was washed off and the plates were photocopied to provide a permanent record of the banding pattern. The distance each band appeared from the origin was measured in mm and alleles were named according to the distance moved relative to the most common allele (Allendorf et al. 1977).

An initial survey for allozyme variation was carried out using one individual from each nest. Thirty-five enzyme systems were investigated (Appendix A). For some enzymes (e. g., alcohol dehydrogenase and esterase) the efficacy of several substrates was tested (Appendix A). Each of the five buffer systems was used in combination with each enzyme system to determine the best running conditions for each enzyme. Next an additional nine spiders from each colony were surveyed for each of the enzymes resolved in the initial screen.

Analysis.—The resulting data on the distribution and frequencies of genotypes were analyzed using Wright's F-statistics (Wright 1951, 1965; Long 1986; Weir 1990; Weir & Cockerham 1984). Both three level and four level analyses were carried out. In three level analyses the total departure from panmixia observed in a population (F_{it}) is partitioned into that due to division of the population into colonies (F_{st}) and that due to non-random mating within colonies (F_{is}). In the four level analyses F_{it} is partitioned into the portion due to division of the population into subpopulations (F_{ct}), colonies within subpopulations (F_{sc}), and F_{is}. In the four level analysis the clusters were the two collection sites and colonies were the subgroups within collection sites.

RESULTS

Of the 35 enzymes screened, 22 yielded scorable bands (Appendix A). The enzymes AAT, MDH, and IDH each produced two bands, presumably representing products of two gene loci, as is true of other organisms (Richardson et al. 1986). The other enzymes appear to be coded for by a single gene locus each. Thus the 22 scorable enzymes are the products of 25 genetic loci.

Two loci showed scorable polymorphisms. Lactate dehydrogenase (LDH) had two alleles, Ldh^{100} and Ldh^6. Glucose-6-phosphate dehydrogenase (G6PDH) had two alleles, $G6pdh^{100}$ and $G6pdh^{84}$. Hexokinase (HK), showed uninterpretable variation and could not be used in this study. LDH was clearest when run in I buffer; G6PDH was clearest in I buffer with the addition of NADP at 1 mM concentration.

LDH and G6PDH genotypes from individuals in each colony are presented in Table 1. The Ldh^6 allele was found only in colonies from the G. K. V. K. campus and was present in all of these colonies. One colony was fixed for Ldh^6, whereas the rest were polymorphic. The allele $G6pdh^{84}$ was found in only one colony on the I. I. S. campus; all members of the colony were homozygous for this allele.

Three level F-statistics were estimated for both LDH and G6PDH (Fig. 1, Table 2). Since Ldh^6 and $G6pdh^{84}$ were each found at only one of the two collecting sites, there may also be subdivision of the population on a scale larger than the individual colony. To quantify this, four level F-statistics were also estimated (Table 2).

DISCUSSION

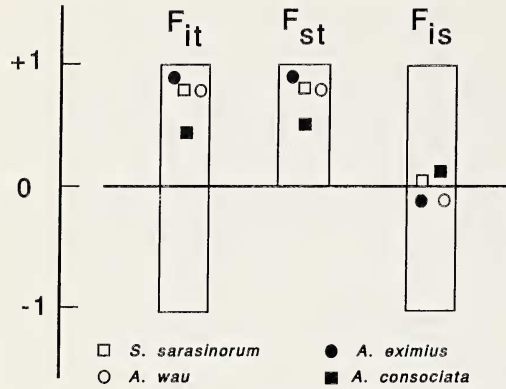In these samples of *S. sarasinorum*, few loci are polymorphic and the majority of colonies

Figure 1.—Plot of mean three level F-statistic values for cooperative spiders. The length of the boxes on the y-axis indicates the range of possible values for F_{it}, F_{st} and F_{it}; the symbols show actual values. Open squares = *Stegodyphus sarasinorum*; filled circles = *Anelosimus eximius*; open circles = *Achaearanea wau*; closed squares = *Agelena consociata*.

consist of identical homozygotes. The high values for F_{it} (Fig. 1) indicate an extreme departure from panmixia in this population. Inspection of Table 1 shows that there is subdivision of the population at two scales: between subpopulations (i. e., collection sites) and among colonies within the two subpopulations. Allele Ldh^6 was found only at the G. K. V. campus, and allele $G6pdh^{84}$ was found only at the I. I. S. campus. Within each subpopulation, further population structure is due to subdivision of the populations into colonies.

Low allozyme diversity in the Bangalore *S. sarasinorum* severely limits our inferences about population structure in this species. Clearly, there is subdivision of the population into clusters and departure from panmixia, as evidenced by the distribution of the LDH alleles. However, the absence of Ldh^6 and the extremely low frequency of $G6pdh^{84}$ at the I. I. S. site means that it is not possible to draw conclusions about dispersal and gene flow among colonies at this site. The presence of two LDH alleles at the G. K. V. campus would make this a better location for study of dispersal and gene flow among colonies than the I. I. S. site. Unfortunately, we collected most samples from the I. I. S. site and only four from the G. K. V. site.

Allozyme electrophoresis has been used in studies of three other cooperative spiders: *Achaearanea wau* Levi (Theridiidae) (Lubin & Crozier 1985), *A. eximius* (Keyserling) (Theri-

Table 1.—Genotype counts for the lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) loci in 29 colonies of *Stegodyphus sarasinorum*. SS, homozygote for the slow allele; FF, homozygote for the fast allele; SF, heterozygote. Ldh^{100} = F, Ldh^6 = S, $G6pdh^{100}$ = F, $G6pdh^{84}$ = S.

Colony	LDH			G6PDH		
	SS	SF	FF	SS	SF	FF
G. K. V. K. Agricultural University Campus						
A1&2	8	16	12	0	0	2
B1	9	2	3	0	0	4
B2	13	0	0	0	0	0
E1	13	8	0	0	0	5
Indian Institute of Science Campus						
C1	0	0	10	0	0	10
C5	0	0	7	0	0	2
C13	0	0	13	0	0	2
C16	0	0	14	0	0	2
C18	0	0	14	0	0	2
C19	0	0	14	0	0	7
C21	0	0	14	0	0	12
C30	0	0	13	0	0	2
C34	0	0	18	0	0	2
C37	0	0	16	0	0	2
C45	0	0	13	0	0	6
C48	0	0	13	0	0	9
C58	0	0	13	0	0	2
C71	0	0	13	0	0	2
C75	0	0	13	0	0	2
C76	0	0	15	0	0	2
C77	0	0	13	0	0	2
C78	0	0	13	0	0	12
D1	0	0	6	3	0	0
D2	0	0	13	0	0	2
D3	0	0	8	0	0	2
D4a	0	0	13	0	0	2
D4b	0	0	8	0	0	0
D4c	0	0	14	0	0	2
D4d	0	0	12	0	0	3
Total	31	26	328	3	0	102

diidae) (Smith 1986, Smith & Hagen unpublished data), and *Agelena consociata* Denis (Agelenidae) (Roeloffs & Riechert 1988). The low frequency of polymorphic loci in *S. sarasinorum* (2 of 25 loci or 8%) agrees with the levels of variability found in other cooperative spiders. Lubin & Crozier (1985) found 1 of 21 loci (5%) to be polymorphic in *Achaearanea wau* and Smith (1986) and Smith & Hagen (unpublished data) found no more than 3 of 51 loci (6%) to be polymorphic within any population of *Anelosimus eximius* examined. *Agelena consociata* has a higher, but still low, frequency of polymorphic

Table 2.—Three- and four-level F-statistic estimates for cooperative spiders. Number of loci = the number of polymorphic loci on which estimates are based.

Species	Three level F_{it}	F_{st}	F_{is}	# loci	Source
Stegodyphus sarasinorum					
LDH	0.675	0.636	0.108	–	this study
G6PDH	1	1	0	–	this study
Mean	0.838	0.818	0.054	2	this study
Achaearanea wau	0.786	0.804	−0.088	1	Lubin & Crozier 1985
Agelena consociata	0.458	0.517	0.131	5	Roeloffs & Riechert 1988
Anelosimus eximius	0.886	0.885	−0.083	4	Smith 1986

Species	Four level F_{it}	F_{ct}	F_{sc}	F_{is}	# loci	Source
Stegodyphus sarasinorum						
LDH	0.854	0.804	0.164	0.108	–	this study
G6PD	1	1	0	0	–	this study
Mean	0.927	0.902	0.082	0.054	2	this study
Anelosimus eximius (from 3 localities in Suriname)	0.896	0.074	0.890	−0.083	3	unpublished data

loci: Roeloffs & Riechert (1988) found 7 of 22 loci (32%) to be polymorphic in a population of this species. In contrast, Smith (1987) found 22 of 35 loci (63%) to be polymorphic in the subsocial species *Anelosimus studiosus*.

Roeloffs & Riechert (1988), Smith (1986) and Smith & Hagen (unpublished data) calculated three level F-statistics from their genotype data; Lubin & Crozier (1985) presented genotype data which we used to estimate F-statistics (Table 2).

Certain aspects of population structure seem common to all four cooperative social spiders: low frequency of polymorphic enzymes, extreme departure from panmixia, and subdivision of the population into colonies. Other aspects of population structure differ among species. In both *S. sarasinorum* and *A. wau* there is significant genetic differentiation among subpopulations: colonies of the same genotype are clustered. This is different from the situation in *Anelosimus eximius*. A four level F-statistical analysis of *A. eximius* collected from three sites in Suriname separated by more than 50 km showed little or no genetic differentiation among subpopulations; virtually all population subdivision was at the level of colonies (Smith & Hagen unpublished data).

Many questions about *S. sarasinorum* remain unanswered by these data. The fact that different alleles were present in the two collection sites implies that there are some barriers to gene flow between these populations. The published observations of ballooning in *Stegodyphus* would, on the other hand, suggest good dispersal powers. It is possible that dispersal and mixing takes place on a small scale (i. e., among colonies close to one another) but that populations are isolated by distance or unsuitable habitat. Another possibility is that colonies of *S. sarasinorum* reproduce by means of swarming, as has been reported for *A. wau* (Lubin & Crozier 1985, Levi et al. 1982), so that spiders in nearby colonies are likely to be members of the same large extended family. These need to be answered by sampling additional colonies from regions in which more than one allele for a locus is present, or by turning to other methods, such as nuclear micro-satellite markers (e. g., Queller et al. 1993) to detect genetic variation among individuals in the samples already collected.

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Appendix A.—Enzymes (abbreviation, name, Enzyme Commission number) examined in this study of *Stegodyphus sarasinorum* and the buffer that gave best resolution: NA, no activity in any of the five buffer systems examined; UR, active but unresolved on the five buffer systems examined. Those scorable enzymes indicated by the symbol (2) are considered to be coded by two loci (see text for further explanation).

AAT (2)	aspartate aminotransferase	(2.6.1.1)	B
AC	aconitase	(4.2.1.3)	B
ACP	acid phosphatase	(3.1.3.2)	B
ADA	adenosine deaminase	(3.5.4.4)	B
ADH	alcohol dehydrogenase	(1.1.1.1)	
	with ethanol as substrate		NA
	ODH—with octanol as substrate		NA
	PDH—with pentanol as substrate		NA
AK	adenylate kinase	(2.7.1.20)	C
ALD	aldolase	(4.1.2.13)	B, I
ALDH	aldehyde dehydrogenase	(1.1.1.1)	NA
AO	aldehyde oxidase	(1.2.3.1)	NA
DIA	diaphorase	(1.6.*.*)	NA
EST	esterase	(3.1.1.1)	
	EST-α—with α-naphthyl acetate as substrate		D
	EST-β—with β-naphthyl acetate as substrate		D
	EST-α, β—with α and β-naphthyl acetate as substrate		D, C
	EST*—with methylumbelliferyl acetate as substrate		NA
FDP	fructose-1,6-diphosphatase	(3.1.3.11)	I, D
FUM	fumarase	(4.2.1.2)	NA
αGPD	α-glycerol-3-phosphate dehydrogenase	(1.1.1.8)	NA
GPI	glucose phosphate isomerase	(5.3.1.9)	UR
G6PDH	glucose-6-phosphate dehydrogenase	(1.1.1.49)	I
GSR	glutathione reductase	(1.6.4.2)	NA
HBDH	β-hydroxybutyrate dehydrogenase	(1.1.1.30)	B
HK	hexokinase	(2.7.1.1)	I
IDH (2)	isocitrate dehydrogenase	(1.1.1.42)	C, B
LAP	leucine aminopeptidase	(3.4.11.1)	NA
LDH	lactate dehydrogenase	(1.1.1.27)	I, D
MDH (2)	malate dehydrogenase	(1.1.1.37)	C
ME	malic enzyme	(1.1.1.40)	UR
MPI	mannose phosphate isomerase	(5.3.1.8)	I
NP	purine nucleoside phosphorylase	(2.4.2.1)	NA
PEP	Peptidase	(3.4.11)	NA
	[phe-pro]—with phenylalanine-proline as substrate		
PGD	6-phosphogluconate dehydrogenase	(1.1.1.46)	B, A
P3GDH	3-phosphoglycerate dehydrogenase	(1.1.1.95)	C
PGK	phosphoglycerate kinase	(2.7.2.3)	NA
PGM	phosphoglucomutase	(2.7.5.1)	A
SORDH	sorbitol dehydrogenase	(1.1.1.14)	B
TPI	triose phosphate isomerase	(5.3.1.1)	C, B
XDH	xanthine dehydrogenase		NA
XO	xanthine oxidase	(1.2.3.2)	NA